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High-density lipoprotein subfractions and carotid plaque: The Northern Manhattan Study

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Abstract

Objective—The objective of this cross-sectional analysis was to investigate the relation between two major high-density lipoprotein cholesterol (HDL-C) subfractions (HDL2-C and HDL3-C) and carotid plaque in a population based cohort.

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Methods—We evaluated 988 stroke-free participants (mean age 66±8 years; 40% men; 66% Hispanic and 34% Non-Hispanic) with available data on HDL subfractions using precipitation method and carotid plaque area and thickness assessed by a high-resolution 2D ultrasound. The associations between HDL-C subfractions and plaque measurements were analyzed by quantile regression.

Results—Plaque was present in 56% of the study population. Among those with plaque, the mean±SD plaque area was 19.40±20.46 mm² and thickness 2.30±4.45 mm. The mean±SD total HDL-C was 46±14 mg/dl, HDL2-C 14±8 mg/dl, and HDL3-C 32±8 mg/dl. After adjusting for demographics and vascular risk factors, there was an inverse association between HDL3-C and plaque area (per mg/dl: beta= −0.26 at the 75th percentile, p=0.001 and beta= −0.32 at the 90th percentile, p=0.02). A positive association was observed between HDL2-C and plaque thickness (per mg/dl; beta= 0.02 at the 90th percentile, p=0.003). HDL-C was associated with plaque area (per mg/dl: beta= −0.18 at the 90th percentile, p=0.01), but only among Hispanics.

Conclusion—In our cohort we observed an inverse association between HDL3-C and plaque area and a positive association between HDL2-C and plaque thickness. HDL-C subfractions may have different contributions to the risk of vascular disease. More studies are needed to fully elucidate HDL-C anti-atherosclerotic functions in order to improve HDL-based treatments in prevention of vascular disease and stroke.

Keywords

high-density lipoprotein; high-density lipoprotein subfractions; carotid plaque

Introduction

Epidemiological studies consistently demonstrate that low levels of high density lipoprotein cholesterol (HDL-C) are associated with increased risk for cardiovascular disease (CVD) and stroke^{1, 2}. The same inverse association has been demonstrated between HDL-C and carotid atherosclerosis³, but with less consistency.

However, recent clinical trials have failed to translate the same epidemiological association into evidence that pharmacological raising of HDL-C prevents CVD morbidity and mortality^{4–6}. Such studies reinforce the need to focus on heterogeneity and functionality of HDL-C subclasses, rather than on measurements of total HDL-C, in order to better explain its pleiotropic effects.

Based on its density, HDL-C has two major subfractions, more dense and smaller HDL3-C and less dense and larger HDL2-C cholesterol. High-density lipoprotein subfractions differ in their biochemical properties, vascular metabolism, and biological activity, and their distribution represents a dynamic process that can be altered by the presence of chronic diseases, drug therapies, and lifestyle changes. Decreased risk of CVD has been linked to higher levels of HDL2-C cholesterol, but not consistently, and uncertainty over the value of HDL-C subfractions in vascular risk remains^{7, 8}.

Atherosclerosis is a disease involving a series of inflammatory events. Carotid plaque, as a focal manifestation of atherosclerosis, represents a validated subclinical ultrasonographic

marker of cardiovascular disease and stroke⁹. Similar to the association with CVD, the severity and progression of atherosclerosis has been predominantly linked to lower levels of the HDL2-C subfraction.^{10–12}. However, the benefits of HDL3-C subfraction have also been well documented in the pathogenesis of atherosclerosis^{13–15}. The aim of this study was to investigate the relationship between the HDL2-C and HDL3-C subfractions and carotid plaque in a large and predominantly Hispanic cohort comprised of stroke-free individuals.

Material and Methods

Study Participants

We included 988 stroke-free participants from the Northern Manhattan Study (NOMAS), an ongoing, prospective, population-based study of stroke incidence and vascular risk factors, who had data available on HDL-C, measured by precipitation, subfractions and carotid plaque measured by high-resolution B-mode ultrasound. Details on the subject ascertainment, extensive assessments, and methods used in NOMAS are described elsewhere¹⁶. Briefly, eligible subjects: a) had never been diagnosed with ischemic stroke; b) were >40 years old; and c) resided in Northern Manhattan for ≥ 3 months, in a household with a telephone. Subjects were identified by random-digit dialing. The telephone response rate was 91%. Subjects were recruited from the telephone sample to have an in-person baseline interview and assessment. The enrollment response rate was 75%, the overall participation rate was 69%, and a total of 3298 subjects were enrolled. Of the total of 3298 subjects, 54% of subjects had data on the HDL subfractions (n=1793) and carotid ultrasound measurements (n=1788). These two subcohorts overlapped resulting in 30% of the study participants (n=988) having both HDL subfractions and carotid plaque measurements. NOMAS was approved by the Institutional Review Boards of Columbia University Medical Center and the University of Miami, and all participants gave written informed consent.

Baseline Evaluation

Data were collected through interviews with trained research assistants in English or Spanish. Physical and neurological examinations were conducted by study neurologists. Race-ethnicity was based upon self-identification through a series of questions modeled after the US census and conforming to standard definitions outlined by Directive 15¹⁷. Standardized questions were adapted from the Behavioral Risk Factor Surveillance System by the Centers for Disease Control regarding hypertension, diabetes, smoking, and cardiac conditions¹⁸. Blood pressure (BP) was measured with mercury sphygmomanometers and appropriately-sized cuffs. Hypertension was defined as a BP ≥ 140/90 mmHg (based on the average of two measurements during one sitting), the patient's self-reported hypertension, or use of anti-hypertensive medications. Diabetes mellitus was defined by fasting glucose ≥ 126 mg/dl, the patient's self-reported diabetes, or use of insulin or oral anti-diabetic medication. Body mass index (BMI) was calculated in kg/m². Smoking was categorized as never smoking, former smoking, and current (within the past year) smoking. Mild-moderate alcohol use was defined as current drinking of >1 drink per month and ≤ 2 drinks per day. Physical activity was defined as the frequency and duration of 14 different recreational activities during the 2-week period before the interview, as described previously¹⁶.

HDL-C, HDL2-C and HDL3-C Measurements

Blood samples were drawn after an overnight fast. Plasma levels of cholesterol and triglycerides (TGs) were measured using standardized enzymatic procedures with a Hitachi 705 automated spectrophotometer (Boehringer Mannheim, Mannheim, Germany). HDL-C was measured after precipitation of plasma apo B-containing lipoproteins with phosphotungstic acid. The inter-assay coefficient of variation in our laboratory was 3% for HDL-C. Two major HDL subfractions were determined in plasma by sequential precipitation using heparin-manganese and dextran sulfate¹⁹. In this procedure, apo B-containing lipoproteins are precipitated in the first precipitation reaction using heparin-manganese chloride at final concentrations of 1.26 mg/ml and 0.091 M, respectively. The supernatant (total HDL-C) is removed, an aliquot is saved for analysis, and dextran sulphate (mol wt 15,000; Genzyme, Cambridge, MA) is added to precipitate HDL2-C, which was estimated by subtracting HDL3-C from HDL-C. Following centrifugation, the supernatant (HDL3-C) is removed and analyzed for cholesterol content. The inter-assay coefficients of variation for HDL2-C and HDL3-C assays are 8% and 7%, respectively, at levels of 28 mg/dl (HDL2-C) and 32 mg/dl (HDL3-C).

Carotid Ultrasound

High-resolution B-mode ultrasound (GE LogIQ 700, 9/13-MHz linear-array transducer) was performed by trained and certified sonographers using standardized and validated scanning and reading protocols as described previously²⁰. Plaque is defined as a focal wall thickening or protrusion in the lumen more than 50% greater than the surrounding thickness. Carotid plaque area (mm²) and thickness (mm) were measured using an automated computerized edge tracking software M'Ath (Paris, France)²¹. Total plaque area (TPA) was a sum of area measured in all plaques within an individual. Maximum plaque thickness was measured for each plaque in an individual at the highest plaque prominence between the lumen-intima and media-adventitia boundaries. The maximum value of maximal thickness measured in all plaques within an individual was used in the analyses²².

Statistical Analysis

The primary independent variables of interest were HDL2-C, HDL3-C, and total HDL-C, all assessed as continuous measurements in mg/dl. The HDL-C variables were examined in relation to the demographics, anthropometrics, and lifestyle and vascular risk factors, among 988 NOMAS participants. We calculated the means and standard deviations. Due to the non-normal distribution of plaque area and thickness, with 44% of the study population without plaque, we used quantile regression to examine these plaque phenotypes as continuous outcomes. For individuals without plaque a value of 0 was assigned for each of these plaque phenotypes. For plaque area and thickness we chose the 75th and 90th percentiles as our outcomes of interest. Because a large portion of the study population did not have plaque present, we chose the 75th percentile of plaque thickness and area to represent a middle value of these variables among those that had plaque. The 90th percentile was chosen to represent a value at the high end of our phenotype spectrum. The association between HDL-C variables and plaque presence was examined using logistic regression models.

The associations of total HDL-C, HDL2-C and HDL3-C with all plaque measurements were examined and adjusted for age, sex, race/ethnicity, low density lipoprotein cholesterol (LDL-C), TGs, smoking, body mass index (BMI), diabetes, hypertension, mild-moderate alcohol use, and physical activity. Finally, a stratified analysis by Hispanic ethnicity was performed as well as an analysis of HDL-C subfractions stratified by the levels of total HDL-C (cut-offs: men < 40 mg/dl and women < 50 mg/dl), both as exploratory analyses. SAS version 9.1 (SAS institute, Cary, NC) was used for statistical analyses and $p < 0.05$ was considered significant.

Results

The mean age among the 988 study participants was 66 ± 8 years and 60% were women. Other demographic characteristics and vascular risk factors in relation to HDL variables are presented in Table 1. The mean $\pm$ SD levels of total HDL-C, HDL2-C and HDL3-C were as follows 46 ± 14 mg/dl, 14 ± 8 mg/dl, and 32 ± 8 mg/dl, respectively. The mean total HDL-C was similar in the NOMAS participants with carotid plaque measurements but without available HDL subfractions ($n = 806$; 47 mg/dl), suggesting unbiased selection of our sub-cohort sample.

A strong positive correlation was observed between total HDL-C and each of the HDL-C subfractions (HDL-C:HDL2-C; $r = .81$, $p < 0.0001$ and HDL-C:HDL3-C; $r = .75$, $p < 0.0001$, respectively), as expected. Conversely, a weak positive correlation was observed between HDL2-C and HDL3-C subfractions ($r = .34$, $p < 0.0001$).

Plaque was present in 56% of the study population and among them, the mean plaque area was 19.40 ± 20.46 mm² and the mean maximum plaque thickness was 2.30 ± 4.45 mm. For the analyses of the association between HDL-C variables and plaque measurements we excluded 150 participants (15% of the study population) that were on lipid-lowering medications, the majority of them ($n = 128$) being on statins. Table 2 shows the association between the HDL variables and plaque thickness, area and presence in the fully adjusted model. Total HDL-C was not associated with any of the plaque measurements. HDL3-C subfraction showed an inverse association with the 75th and 90th percentiles of plaque area. The association of HDL3-C with the 75% percentile of plaque area was stronger among the individuals with low HDL-C as compared to those with higher HDL-C levels (per mg/dl; $\beta = -0.38$ mm, $p = 0.02$ vs. $\beta = -0.17$, $p = 0.19$, respectively). Similar to the 75th percentile, an inverse association between HDL3-C and the 90th percentile of plaque area was more pronounced among participants with low HDL-C as compared to the individuals with higher HDL-C levels (per mg/dl; $\beta = -0.38$ mm, $p = 0.12$ vs. $\beta = -0.20$, $p = 0.43$, respectively). HDL2-C was positively associated with the 90th percentile of plaque thickness, while no association was observed between HDL2-C and plaque area. Among the individuals with and without plaque presence, the levels (all in mg/dl) were similar for total HDL-C (mean $\pm$ SD; 46 ± 14 vs. 46 ± 14 , respectively, $p = 0.71$), HDL3-C (mean $\pm$ SD; 31 ± 8 vs. 32 ± 8 , respectively, $p = 0.21$), and HDL2-C (mean $\pm$ SD; 14 ± 8 vs. 14 ± 8 , respectively, $p = 0.93$). None of the HDL-C variables were associated with plaque presence.

Stratified analyses of all HDL-C variables in relation to plaque thickness and area by Hispanic ethnicity are shown in Table 3. Only among Hispanics HDL3-C was inversely associated with the 90th percentile of plaque thickness and both total HDL-C and HDL3-C were inversely associated with the 90th percentile of plaque area. We did not observe an association between HDL2-C and any of the plaque measurements in this stratified analysis. There was some suggestion of potential effect modification by ethnicity for the relationships between total HDL-C and HDL3-C with the 90th percentile of plaque thickness. However, the limited statistical power of the tests for interaction as well as the main effects in the exploratory stratified analyses is important to note, and therefore these results should be interpreted with caution.

Discussion

In the present study we report an association between increased levels of HDL3-C with smaller plaque area. In contrast, we observed a positive association between HDL2-C and plaque thickness. In addition to HDL3-C, elevated levels of total HDL-C were associated with reduced plaque thickness, but only among Hispanics. The same association was stronger for HDL3-C than for total HDL-C. Our study was mostly comprised of Caribbean Hispanics and therefore further studies are needed to confirm the race-ethnic effect modification of HDL3-C and total HDL-C relationship with plaque burden among other diverse populations.

An inverse association between total HDL-C and the risk of CVD has been shown in multiple epidemiological studies²³⁻²⁵. However, total HDL-C concentration measurement does not offer insight into the variable composition of different HDL-C subfractions responsible for complex functions and atheroprotective mechanisms associated with cardiometabolic outcomes²⁶. The complexity of HDL-C subclass proportions and activities can be altered from “functional” to “dysfunctional” under a variety of metabolic abnormalities and pharmacological and non-pharmacological treatments. More importantly, their heterogeneous properties are frequently unrelated to the overall HDL-C concentration and remain undetected by measurement of total HDL-C²⁷.

Several studies have examined differential effects of HDL-C and HDL subfractions on markers of atherosclerosis with controversial results. However, there are currently several methods using different approaches in analyzing physical and chemical properties of HDL²⁸. Therefore, it is challenging to compare data obtained with different methodology in order to evaluate the relationship of HDL subfractions and their functions with CVD risk. Three studies reported similar findings by using different laboratory methods. Total HDL-C and HDL2-C subfractions quantified by ultracentrifugation, have been correlated inversely with the coronary artery disease score in 105 male survivors of myocardial infarction under the age 45¹¹. However, the association was only significant for HDL2-C subfraction, indicating its protective effect on the progression of coronary atheromatosis. Another study, using the gradient gel electrophoresis method,¹⁰ examining the effect between five major HDL subclasses and coronary atherosclerosis, has reported similar findings. According to that study, the protective effects of total HDL-C for coronary heart disease or coronary atherosclerosis may be accounted by larger HDL2b-C particles rather than smaller HDL3-C

particles. Similarly, Xu et al¹² have reported that HDL2-C and HDL3-C subfractions, determined by two dimensional gel electrophoresis, are highly correlated with coronary stenosis, but with HDL3a-C and HDL3b-C exhibiting positive and HDL2b-C negative correlation.

However, a more recent study¹⁵ reported that HDL3-C, compared to total HDL-C, had a stronger inverse association with plaque lipid-rich necrotic core, a high-risk feature of atherosclerotic risk. Our study, using a precipitation method, supports these results, suggesting that HDL3-C is more strongly inversely associated with carotid plaque burden than HDL2-C and total HDL-C.

The benefits of HDL3-C subfraction have been well documented in the pathogenesis of atherosclerosis. Small and dense HDL3-C subfraction is less prone to oxidation due to greater paraoxonase 1 activity¹³ and it appears to inhibit LDL oxidation to a greater extent than HDL2-C¹⁴. In addition, HDL3-C has been more effective than HDL2-C in inhibiting vascular cell adhesion molecule expression in endothelial cells²⁹, involved in mediating the process of early atherosclerosis. The benefits of HDL3-C have also been recently extended to increased longevity³⁰, as well as reduced risk of cancer in men³¹. Also, smaller and more dense HDL3-C subfraction represents the major subfraction in newborns, thus providing a greater protection against infection in early life³².

Despite longstanding evidence from epidemiological studies for the inverse relationship between total HDL-C and CVD risk, the most recent pharmacological strategies to raise HDL-C have failed to yield positive results. The clinical trials with torcetrapib, a cholesteryl ester transfer protein (CETP) inhibitor, aimed at raising total HDL-C, concomitant with reduction in low-density lipoprotein cholesterol, did not translate into reduced CVD morbidity and mortality^{4, 33, 34}. A more recent study reported that the same CETP inhibitor, combined with atorvastatin, enhanced the capacity of postprandial HDL2a-C and HDL2b-C to mediate cellular free cholesterol efflux. The same mechanism was superior for larger HDL2-C than HDL3-C subspecies³⁵. Thus, the association between total HDL-C and subclinical and clinical CVD seems complex. A better understanding of HDL-C subfractions may be important for more effective prevention and treatment of CVD.

Lastly, carotid plaque as a distinct phenotype of atherosclerosis is more influenced by environmental and less by genetic factors²¹. Furthermore, plaque grows longitudinally along the carotid axis of flow more than two times faster than thickness³⁶. In our analysis HDL3-C, with its potent anti-oxidative and anti-inflammatory properties, was more inversely associated with plaque area, as a more sensitive measure of atherosclerotic burden than thickness³⁷. In regard to ethnic differences in non-invasive measures of atherosclerosis, several studies have shown the significant association between the presence of carotid plaque and the risk of vascular events but predominantly in white populations. In our current analysis, the association between total HDL-C and the 90th percentile of plaque area, as well as the associations between HDL3-C and the 90th percentiles of plaque thickness and area were present only among Hispanics, though statistically significant interactions with Hispanic ethnicity were not found in these power-limited analyses. We have previously shown that even though Hispanics had lower amount of plaque thickness, compared to

whites and blacks, they were more susceptible to vascular events. More specifically, Hispanics with carotid plaque had a three- to fourfold increased risk of vascular events in comparison to a one- to two-fold increased risk among Non-Hispanics²².

Strengths of the current study include a large population with extensive information obtained on vascular risk factors. We used a dual-step precipitation method for HDL-C isolation. This method has a greater precision than the electrophoresis method (the coefficient of variance of the within-run analysis; 4.8% vs. 10.7%, respectively)³⁸ used in some of the previous studies examining the association between HDL-C subfractions and carotid plaque atherosclerotic characteristics^{10, 12}. Our research also contributes to greater understanding of the association between total HDL-C and its subfractions with atherosclerotic plaque development. One of the potential limitations of our study is its cross-sectional design. We could not assess the changes in the levels of HDL subfractions and whether they affected progression of atherosclerosis over time. Finally, we assessed the plasma levels of two major HDL subfractions without studying their subclasses and the biological activity responsible for diverse atheroprotective properties.

In summary, our study shows that the HDL3-C subfraction, unlike HDL2-C and total HDL-C, is inversely associated with plaque area as a marker of subclinical atherosclerosis among stroke-free individuals. These novel findings support the role of HDL-C subfractions in atherosclerosis and emphasize the overall complexity of HDL-C functions. Future studies are needed to fully elucidate HDL-C anti-atherosclerotic functions, and their role among different ethnic groups, in order to improve HDL-based treatments in prevention of CVD and stroke.

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Reference List

1. Sacco RL, Benson RT, Kargman DE, et al. High-density lipoprotein cholesterol and ischemic stroke in the elderly: the Northern Manhattan Stroke Study. *JAMA*. 2001 Jun 6; 285(21):2729–35. [PubMed: 11386928]
2. Maron DJ. The epidemiology of low levels of high-density lipoprotein cholesterol in patients with and without coronary artery disease. *Am J Cardiol*. 2000 Dec 21; 86(12A):11L–4L.
3. Amarenco P, Labreuche J, Touboul PJ. High-density lipoprotein-cholesterol and risk of stroke and carotid atherosclerosis: a systematic review. *Atherosclerosis*. 2008 Feb; 196(2):489–96. [PubMed: 17923134]
4. Bots ML, Visseren FL, Evans GW, et al. Torcetrapib and carotid intima-media thickness in mixed dyslipidaemia (RADIANCE 2 study): a randomised, double-blind trial. *Lancet*. 2007 Jul 14; 370(9582):153–60. [PubMed: 17630038]
5. Barter P, Kastelein J, Nunn A, Hobbs R. High density lipoproteins (HDLs) and atherosclerosis; the unanswered questions. *Atherosclerosis*. 2003 Jun; 168(2):195–211. [PubMed: 12801602]
6. Voight BF, Peloso GM, Orho-Melander M, et al. Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. *Lancet*. 2012 Aug 11; 380(9841):572–80. [PubMed: 22607825]

7. Superko HR, Pendyala L, Williams PT, Momary KM, King SB III, Garrett BC. High-density lipoprotein subclasses and their relationship to cardiovascular disease. *J Clin Lipidol*. 2012 Nov; 6(6):496–523. [PubMed: 23312047]
8. Akinkuolie AO, Paynter NP, Padmanabhan L, Mora S. High-density lipoprotein particle subclass heterogeneity and incident coronary heart disease. *Circ Cardiovasc Qual Outcomes*. 2014 Jan 1; 7(1):55–63. [PubMed: 24248942]
9. Della-Morte DGF, Palmirotta R, Testa G, et al. Genetics of ischemic stroke, stroke-related risk factors, stroke precursors and treatments. *Pharmacogenomics*. 2012; 13(5):595–613. [PubMed: 22462751]
10. Johansson J, Carlson LA, Landou C, Hamsten A. High density lipoproteins and coronary atherosclerosis. A strong inverse relation with the largest particles is confined to normotriglyceridemic patients. *Arterioscler Thromb*. 1991 Jan; 11(1):174–82. [PubMed: 1987996]
11. Hamsten A, Walldius G, Szamosi A, Dahlen G, de FU. Relationship of angiographically defined coronary artery disease to serum lipoproteins and apolipoproteins in young survivors of myocardial infarction. *Circulation*. 1986 Jun; 73(6):1097–110. [PubMed: 3698244]
12. Xu YX, Fu MD, Xu YH, Yang LC. Relationship between the contents of serum HDL subclasses and the extent of coronary stenosis in coronary heart disease patients. *Sichuan Da Xue Xue Bao Yi Xue Ban*. 2004 Jul; 35(4):500–2. [PubMed: 15291110]
13. Shuhei N, Soderlund S, Jauhiainen M, Taskinen MR. Effect of HDL composition and particle size on the resistance of HDL to the oxidation. *Lipids Health Dis*. 2010; 9:104. [PubMed: 20863394]
14. Kontush A, Chantepie S, Chapman MJ. Small, dense HDL particles exert potent protection of atherogenic LDL against oxidative stress. *Arterioscler Thromb Vasc Biol*. 2003 Oct 1; 23(10):1881–8. [PubMed: 12920049]
15. Phan BA, Chu B, Polissar N, Hatsukami TS, Yuan C, Zhao XQ. Association of high-density lipoprotein levels and carotid atherosclerotic plaque characteristics by magnetic resonance imaging. *Int J Cardiovasc Imaging*. 2007 Jun; 23(3):337–42. [PubMed: 17086362]
16. Sacco RL, Gan R, Boden-Albala B, et al. Leisure-time physical activity and ischemic stroke risk: the Northern Manhattan Stroke Study. *Stroke*. 1998 Feb; 29(2):380–7. [PubMed: 9472878]
17. Wallman KK, Hodgdon J. Race and ethnic standards for Federal statistics and administrative reporting. *Stat Report*. 1977; 77–110:450–4.
18. Sacco RL, Elkind M, Boden-Albala B, et al. The protective effect of moderate alcohol consumption on ischemic stroke. *JAMA*. 1999 Jan 6; 281(1):53–60. [PubMed: 9892451]
19. Hirano T, Nohtomi K, Koba S, Muroi A, Ito Y. A simple and precise method for measuring HDL-cholesterol subfractions by a single precipitation followed by homogenous HDL-cholesterol assay. *J Lipid Res*. 2008 May; 49(5):1130–6. [PubMed: 18223297]
20. Rundek T, Blanton SH, Bartels S, et al. Traditional Risk Factors Are Not Major Contributors to the Variance in Carotid Intima-Media Thickness. *Stroke*. 2013 May 23.
21. Kuo F, Gardener H, Dong C, et al. Traditional cardiovascular risk factors explain the minority of the variability in carotid plaque. *Stroke*. 2012 Jul; 43(7):1755–60. [PubMed: 22550054]
22. Rundek T, Arif H, Boden-Albala B, Elkind MS, Paik MC, Sacco RL. Carotid plaque, a subclinical precursor of vascular events: the Northern Manhattan Study. *Neurology*. 2008 Apr 1; 70(14):1200–7. [PubMed: 18354078]
23. Vergeer M, Holleboom AG, Kastelein JJ, Kuivenhoven JA. The HDL hypothesis: does high-density lipoprotein protect from atherosclerosis? *J Lipid Res*. 2010 Aug; 51(8):2058–73. [PubMed: 20371550]
24. Castelli WP. Epidemiology of coronary heart disease: the Framingham study. *Am J Med*. 1984 Feb 27; 76(2A):4–12. [PubMed: 6702862]
25. Touboul PJ, Labreuche J, Bruckert E, et al. HDL-C, triglycerides and carotid IMT: A meta-analysis of 21,000 patients with automated edge detection IMT measurement. *Atherosclerosis*. 2014 Jan; 232(1):65–71. [PubMed: 24401218]
26. Warnick GR. High-density lipoproteins: the neglected stepchildren whose importance as a risk factor continues to be defined. *Clin Chem*. 2008 May; 54(5):923–4. [PubMed: 18443180]
27. Navab M, Anantharamaiah GM, Fogelman AM. The role of high-density lipoprotein in inflammation. *Trends Cardiovasc Med*. 2005 May; 15(4):158–61. [PubMed: 16099381]

28. Rosenson RS, Brewer HB Jr, Chapman MJ, et al. HDL measures, particle heterogeneity, proposed nomenclature, and relation to atherosclerotic cardiovascular events. *Clin Chem*. 2011 Mar; 57(3): 392–410. [PubMed: 21266551]
29. Ashby DT, Rye KA, Clay MA, Vadas MA, Gamble JR, Barter PJ. Factors influencing the ability of HDL to inhibit expression of vascular cell adhesion molecule-1 in endothelial cells. *Arterioscler Thromb Vasc Biol*. 1998 Sep; 18(9):1450–5. [PubMed: 9743234]
30. Williams PT. Low high-density lipoprotein 3 reduces the odds of men surviving to age 85 during 53-year follow-up. *J Am Geriatr Soc*. 2012 Mar; 60(3):430–6. [PubMed: 22329432]
31. Fagot-Campagna A, Knowler WC, Narayan KM, Hanson RL, Saaddine J, Howard BV. HDL cholesterol subfractions and risk of developing type 2 diabetes among Pima Indians. *Diabetes Care*. 1999 Feb; 22(2):271–4. [PubMed: 10333944]
32. Kherkeulidze P, Johansson J, Carlson LA. High density lipoprotein particle size distribution in cord blood. *Acta Paediatr Scand*. 1991 Aug; 80(8–9):770–9. [PubMed: 1957594]
33. Nissen SE, Tardif JC, Nicholls SJ, et al. Effect of torcetrapib on the progression of coronary atherosclerosis. *N Engl J Med*. 2007 Mar 29; 356(13):1304–16. [PubMed: 17387129]
34. Barter PJ, Caulfield M, Eriksson M, et al. Effects of torcetrapib in patients at high risk for coronary events. *N Engl J Med*. 2007 Nov 22; 357(21):2109–22. [PubMed: 17984165]
35. Bellanger N, Julia Z, Villard EF, et al. Functionality of postprandial larger HDL2 particles is enhanced following CETP inhibition therapy. *Atherosclerosis*. 2012 Mar; 221(1):160–8. [PubMed: 22265126]
36. Barnett PA, Spence JD, Manuck SB, Jennings JR. Psychological stress and the progression of carotid artery disease. *J Hypertens*. 1997 Jan; 15(1):49–55. [PubMed: 9050970]
37. Johnsen SH, Mathiesen EB. Carotid plaque compared with intima-media thickness as a predictor of coronary and cerebrovascular disease. *Curr Cardiol Rep*. 2009 Jan; 11(1):21–7. [PubMed: 19091171]
38. Tsai LY, Peng MR, Tsai SM, Hsieh SF. The determination of high density lipoprotein cholesterol separated by electrophoresis and sodium phosphotungstate/Mg²⁺ precipitation: a physical evaluation. *Gaoxiong Yi Xue Ke Xue Za Zhi*. 1989 Sep; 5(9):498–504. [PubMed: 2607561]

Highlights

- Recent trials have challenged the HDL-Cholesterol anti-atherosclerotic hypothesis
- HDL3 is inversely associated with plaque area and plaque thickness
- HDL-C subfractions may have different contributions to the risk of vascular disease
- Investigate HDL “quality” and not only HDL “quantity”

Table 1

Clinical characteristics of study participants

Demographics	N (988)	HDL-C (mg/dl) Mean (SD)	HDL2-C (mg/dl) Mean (SD)	HDL3-C (mg/dl) Mean (SD)
<i>Race/ethnicity</i>				
Hispanic	655	43 (12) *	13 (7) *	30 (7) *
Non-Hispanic	333	48 (15)	16 (9)	33 (9)
<i>Age</i>				
40–59	283	44 (13) *	13 (7) *	31 (8)
60–69	407	45 (14)	14 (8)	31 (7)
70–79	230	47 (15)	15 (9)	32 (9)
80+	68	48 (13)	16 (8)	32 (7)
<i>Sex</i>				
Male	397	41 (13) *	12 (7) *	29 (7) *
Female	591	48 (14)	15 (8)	33 (8)
<i>BMI</i>				
Normal	240	50 (16) *	16 (9) *	33 (8) *
Overweight	457	45 (14)	14 (8)	31 (8)
Obese	288	44 (12)	13 (7)	31 (7)
<i>Smoking</i>				
Current	152	44 (13)	13 (8)	30 (8)
Former	366	45 (14)	14 (9)	31 (8)
Never	470	46 (13)	14 (8)	32 (8)
<i>Mild-moderate alcohol use</i>				
Yes	381	47 (15) *	14 (8)	32 (8) *
No	607	45 (13)	14 (8)	31 (8)
<i>Moderate-heavy physical activity</i>				
Yes	95	49 (15) *	16 (9) *	33 (9) *
No	890	45 (14)	14 (8)	31 (8)
<i>Cholesterol lowering medications</i>				
Yes	150	47 (14)	14 (7)	33 (8)
No	838	45 (14)	14 (8)	31 (8)
<i>Hypertension</i>				
Yes	716	45 (14)	14 (8)	31 (8)
No	272	47 (14)	15 (9)	32 (8)
<i>Diabetes</i>				
Yes	203	43 (13) *	13 (8) *	30 (7) *
No	785	46 (14)	14 (8)	32 (8)
<i>LDL-C (mg/dl)</i>				
>160	818	46 (14) *	14 (8) *	32 (8)
160	166	44 (11)	12 (6)	32 (7)

Demographics	N (988)	HDL-C (mg/dl) Mean (SD)	HDL2-C (mg/dl) Mean (SD)	HDL3-C (mg/dl) Mean (SD)
<i>TGs (mg/dl)</i>				
>150	276	39(10) *	11(6) *	28(7) *
150	711	48(14)	15 (8)	33(8)

* ANOVA or t-test $p < 0.05$ (categories of covariates were compared for the HDL variables as continuous measures).

HDL-C, high-density lipoprotein cholesterol; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; TGs, triglycerides

Table 2

The association of HDL variables with plaque thickness (PT) and plaque area (PA) percentiles and plaque presence

Per mg/dl	PT (mm) (effects, p values)	PA (mm ²) (effects, p values)	Plaque presence OR (95% CI)		
	75%	90%	75%	90%	
HDL-C	0.002	0.01	-0.09	-0.08	1.00
mg/dl	0.71	0.08	0.12	0.35	(0.99-1.01)
HDL2-C	0.02	0.02	0.06	0.13	1.00
mg/dl	0.29	0.003	0.61	0.40	(0.98-1.02)
HDL3-C	-0.01	-0.01	-0.26	-0.32	0.99
mg/dl	0.15	0.35	0.001	0.02	(0.97-1.02)

Quantile regression: adjusted for age, sex, race/ethnicity, LDL-C, and TGs, cholesterol-lowering medication use, smoking, BMI, diabetes, hypertension, mild-moderate alcohol use, physical activity. HDL-C, high-density lipoprotein cholesterol

Table 3

HDL variables and plaque thickness (PT) and plaque area (PA) stratified by ethnicity

Per mg/dl	Non-Hispanic (n=279) (effects, p values)	Hispanic (n=552) (effects, p values)	Interaction p-value
PT mm 75 th percentile			
HDL-C	0.01, 0.12	−0.001, 0.81	0.17
HDL2-C	0.02, 0.16	0.01, 0.57	0.46
HDL3-C	−0.01, 0.56	−0.01, 0.17	0.54
PT mm 90 th percentile			
HDL-C	0.01, 0.15	−0.01, 0.34	0.02
HDL2-C	0.02, 0.37	0.004, 0.74	0.19
HDL3-C	0.003, 0.81	−0.02, 0.03	0.09
PA mm ² 75 th percentile			
HDL-C	0.01, 0.92	−0.08, 0.17	0.46
HDL2-C	0.27, 0.24	−0.06, 0.64	0.13
HDL3-C	−0.37, 0.06	−0.09, 0.35	0.31
PA mm ² 90 th percentile			
HDL-C	0.23, 0.28	−0.18, 0.01	0.36
HDL2-C	0.57, 0.28	0.02, 0.91	0.89
HDL3-C	−0.25, 0.50	−0.39, 0.01	0.52

Quantile regression: adjusted for age, sex, LDL-C, TGs, cholesterol-lowering medication use, smoking, BMI, diabetes, hypertension, mild-moderate alcohol use, physical activity. HDL-C, high-density lipoprotein cholesterol.